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Evidence for Two Distinct Cross-Bridge Populations in Tetanized Frog Muscle Fibers Stretched with Moderate Velocities

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When tetanized frog skeletal muscle fibers are subjected to moderate-velocity stretches (<1 L_0/s), the tension developed above the level of isometric tension starts to decay after a sudden reduction of stretch velocity by more than 40-50%, though the fibers are still being stretched. We analysed the decay of tension response caused by the sudden reduction of stretch velocity, by applying three different types of stretch to a tetanized fiber, i.e., a 1.5% stretch with velocity V_1 (stretch-1), a 1.5% stretch with velocity $V_2 < V_1$ (stretch-2), and a 3% stretch consisting of stretch-1 and stretch-2 applied in succession (stretch-3) and comparing the corresponding tension responses, TR-1, TR-2, and TR-3. It was found that TR-3 to stretch-3 resulted from algebraical summation of TR-1 to the preceding stretch-1 and TR-2 to the subsequent stretch-2. These results can be accounted for by assuming two distinct cross-bridge populations in stretched fibers. © 1998 Academic Press

It is well known that, when a tetanized skeletal muscle is stretched with a moderate velocity, the tension in it rises to a peak at the completion of stretch, and then decays with time (1). The mechanism underlying the tension response to stretch has not yet been well explained in terms of cross-bridges, which extend from the thick filament and interact with the thin filament to produce contraction. During an applied stretch, both the rate of heat production and the rate of ATP-breakdown are reduced below the level of isometric contraction (2, 3), indicating the reduced cycling rate of actin-myosin interaction coupled with ATP hydrolysis. On this basis, it has been suggested that, if the cross-bridges attached to the thin filament are displaced in the direction opposite to that of muscle shortening, they tend to be detached from the thin filament without hydrolysing ATP to build up tension above the isometric level (4-6). Since the tension response to stretch is of fundamental importance for the function of skeletal muscle in the animal body, experimental work is desired to investigate mechanisms underlying the tension response to stretch at the level of cross-bridges.

With respect to properties of the tension response to stretch, Sugi (6) made an observation that, during a moderate-velocity stretch of a tetanized frog muscle fiber, the tension developed above the level of steady isometric tension showed a tendency to decay after a sudden reduction of stretch velocity, though the fiber was still being stretched. In the experiments reported here, we have analysed the tension decay after a sudden reduction of stretch velocity, and found that, after a sudden reduction of stretch velocity by more than 40-50%, the subsequent tension response started on the falling baseline tension equal to the tension decay after the completion of the initial stretch. The algebraical summation of the component tension responses can be accounted for in terms of two distinct cross-bridge populations in the fiber mechanically acting in parallel with each other.

METHODS

Muscle fiber preparation. Single intact muscle fibers (diameter, 90-150 μm; slack length 7-11 mm) were dissected from the semitendinosus muscles of the frog *Rana japonica*, with a pair of aluminium foil connectors attached to both tendons, and mounted horizontally in an experimental chamber (3 ml) filled with Ringer solution containing (mM): 115 NaCl, 2.5, KCl, 1.8 $CaCl_2$, and 10 Tris-maleate (pH 7.2); the two tendinous ends of the fiber were attached to a servomotor (G-100PD, General Scanning) and a tension transducer (AE-801, Senso-Nor) with the connectors, respectively. The fiber was kept at slack length (L_0), and the sarcomere length was adjusted to 2.0 μm by light diffraction with He-Ne laser light. The fibers were tentanized by applying supramaximal 1 ms current pulses (50 Hz) through a multi-electrode assembly.

Experimental apparatus and procedure. The force transducer had a compliance of 0.1 mm/N and a resonant frequency of ca. 5 kHz. The servo-motor was operated by a driving amplifier (JCX-101, General Scanning), and its motor arm position was sensed by a differential transformer. The driving amplifier was controlled by

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a waveform generator (2414A, Biomation) and a timing controller (SS-302J, Nihon Kohden). The temperature of Ringer solution in the chamber was kept at 4°C.

The fiber was first tetanized isometrically to develop steady isometric tension, and then stretched with moderate velocities (≤ 1 L_0/s). Since the magnitude of stretch never exceeded 3% L_0, all experiments were performed within the range of fiber length, where the resting tension was negligible, while the number of the cross-bridges that could interact with the thin filament was always maximum (7). The fibers were tetanized for 1.5 s at intervals of 2-3 min. The length and tension changes of the fibers were stored in the digital wave memory of a digital oscilloscope (type 3091, Nicolet), and displayed on an X-Y recorder or transferred to a microcomputer (type 9650, COMPAQ) for data processing and analysis.

Recording of fiber segment length changes. To ascertain whether each fiber segment was stretched uniformly along the entire fiber length, fine carbon particles were firmly attached to the fiber surface to divide the fiber into several segments. The image of the whole fiber was observed under a Nikon dissecting microscope, and recorded with a high-speed video system (FASTCAM-rabbit 1, Photoron) at 240 frames/s during the course of an applied stretch. Length changes of six to eight consecutive fiber segments along the entire fiber length were measured on the monitor screen with a video microscaler (IV-500, For-A).

RESULTS

General features of tension responses after a reduction of stretch velocity. In agreement with the previous report (6), the tension responses of tetanized frog muscle fibers to moderate-velocity stretches exhibited prominent transient decay after a sudden reduction of stretch velocity by more than 40-50%. Figure 1 shows typical length and tension records in the experiments, in which a tetanized fiber was first stretched by 1.5% L_0 with velocity V_1 and then further stretched by 1.5% L_0 with velocity $V_2 < V_1$. In all cases, the tension first rose to a peak which was reached at the completion of the initial stretch with velocity V_1, and then started to decay after a sudden reduction of stretch velocity to V_2, though the subsequent stretch still went on. The tension, which had decayed after a reduction of stretch velocity, eventually rose again until the completion of the subsequent stretch. Both the magnitude and duration of the tension decay after a sudden reduction of stretch velocity increased with increasing extent of reduction of stretch velocity. If the extent of sudden reduction of stretch velocity was made smaller than 40-50%, the transient decay of tension response became less prominent and eventually disappeared.

As shown in Fig. 1E, the segmental length changes along the entire length of tetanized fiber were almost similar in time course to the applied stretch recorded at the moving fiber end, indicating that tension responses do not result from nonuniform length changes along the fiber, but originate from the basic properties of the cross-bridges in each sarcomere. Similar results were obtained from six other preparations examined.

Algebraical summation of component tension responses after reduction of stretch velocity. The stretches used in the experiments of Fig. 1 are regarded as the sum of the two component stretches of the same magnitude, i.e. the initial stretch with velocity V_1 (stretch-1) and the subse-

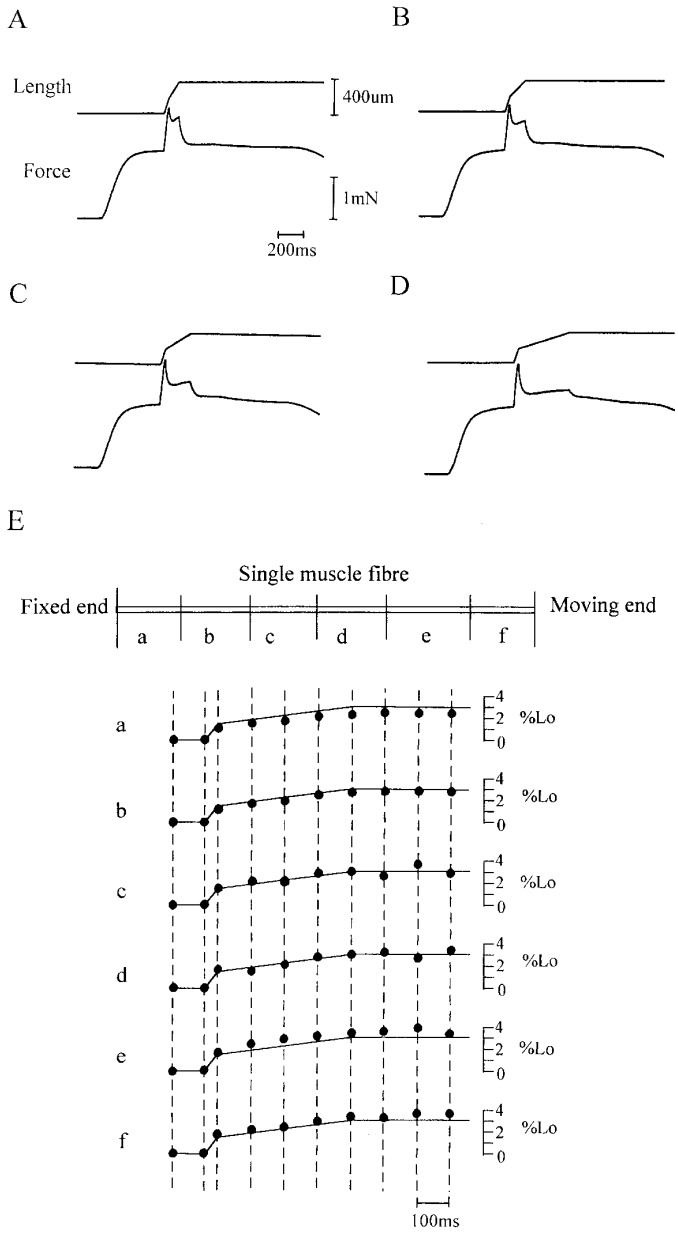

FIG. 1. (A-D) Typical length (upper traces) and tension (lower traces) records showing transient decay of tension response after a sudden reduction of stretch velocity in a tetanized frog muscle fiber. After the development of steady isometric tension, the fiber was first stretched by 1.5% L_0 with velocity V_1 (0.38 L_0/s), and then further stretched by 1.5% L_0 with velocity V_2 (0.5 V_1 in A, 0.25 V_1 in B, 0.1 V_1 in C, and 0.05 V_1 in D). (E) Segmental length changes of a tetanized muscle fiber during a 3% stretch consisting of the initial 1.5% stretch with velocity V_1 (0.38 L_0/s) and the subsequent 1.5% stretch with velocity V_2 (0.1 V_1). Six consecutive fiber segments are designated a-f (inset). Length changes of the segments are expressed in percentage of L_0. The continuous line in each record show the fiber length change at the moving fiber end.

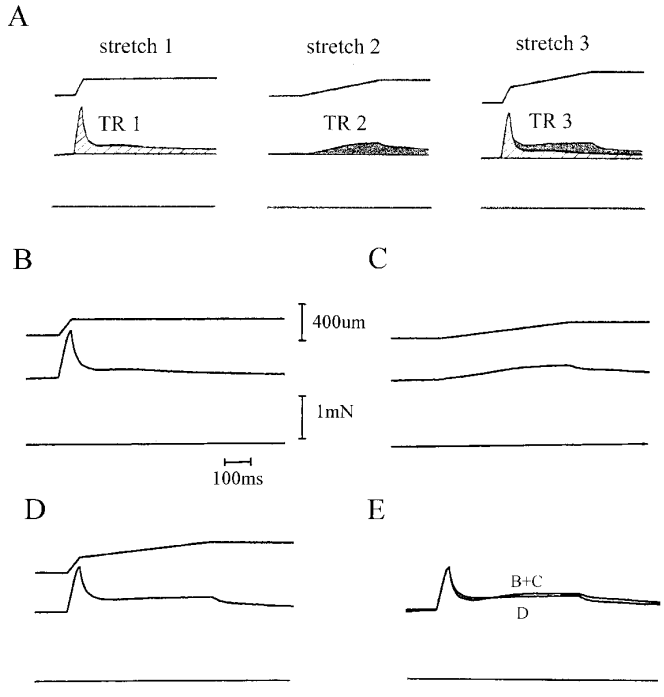

FIG. 2. Algebraical summation of the component tension responses after a sudden reduction of stretch velocity. (A) Diagrams illustrating the component stretches (stretch-1 and stretch-2) and the corresponding component tension responses (TR-1 and TR-2). (B) Tension response to a 1.5% stretch with velocity V_1 (0.38 L_0/s). (C) Tension response to a 1.5% stretch with velocity V_2 (0.1 V_1). (D) Tension response to a 3% stretch consisting of a 1.5% stretch with velocity V_1 immediately followed by a 1.5% stretch with velocity V_2. (E) The tension response in D is superimposed on the tension response constructed by adding the tension responses in C to that in B in the manner illustrated diagrammatically in A.

quent stretch with velocity V_2 (stretch-2), as illustrated in Fig. 2A. Tension response to stretch-1 alone and that to stretch-2 alone will be designated TR-1 and TR-2, respectively, while the tension response to stretches 1 and 2 applied in succession (stretch-3) is designated TR-3. Then, if the two component tension responses (TR-1 and TR-2) sum algebraically to build up TR-3, the transient tension decay after a sudden reduction of stretch velocity (Fig. 1) may result from that TR-2 starts on the falling baseline tension corresponding to the tension decay of TR-1 after the completion of stretch-1. Since both the rate and the magnitude of tension development during a moderate-velocity stretch is smaller, the slower the stretch velocity (1, 6, 8; see also Fig. 2B, C), TR-2 developing on the same falling baseline tension becomes less distinct with smaller V_2 value, as can be actually seen in Fig. 1.

To confirm the algebraical summation of the component tension responses after a reduction of stretch velocity, we recorded TR-1 to stretch-1 alone and TR-2 to stretch-2 alone separately, and then the two tension responses were added algebraically in such a way that TR-2 starts after the completion of stretch-1. As shown in Fig. 2B-E. The tension response TR-3, constructed by adding TR-2 (Fig. 2C) to TR-1 (Fig. 2B) after the completion of stretch-1 was found to be identical with the actual tension response to stretch-3 (Fig. 2D, E). The algebraical summation of the component tension responses was also observed in the other tension responses shown in Fig. 1A-C. Similar results were obtained in six other experiments with a wide range of V_1 (0.1-0.8 L_0/s) and V_2 (0.01 to 0.5 L_0/s). The algebraical summation of the component tension responses was no longer observed if the extent of reduction of stretch velocity was below 40-50%; the tension response, obtained by subtracting TR-1 from the actual tension response, became larger in magnitude than TR-2 recorded by applying stretch-2 alone.

DISCUSSION

The present experiments have shown that, after a sudden reduction of stretch velocity by more than 40-50% during a moderate-velocity stretch of a tetanized frog muscle fiber, the tension response to the subsequent stretch starts on the falling baseline tension equal to the tension decay after the completion of the initial stretch. Similar algebraical summation of the component tension responses has been observed in Ca^{2+}-activated glycerinated fibers of rabbit psoas muscle, though the tension decay is much less prominent than in tetanized frog fibers because of much slower rate of tension decay after the completion of stretch (9). The algebraical summation of the component tension responses implies the presence of two independent tension-generating elements within a tetanized fiber, and can be qualitatively accounted for by assuming two distinct populations of cross-bridges, mechanically acting in parallel with each other, so that the tension generated by the two cross-bridge populations sum algebraically.

On this basis, a possible explanation for the present results is as follows: (i) In an isometrically tetanized fiber, all the cross-bridges interacting with the thin filament are repeating attachment-detachment cycles coupled with ATP hydrolysis. This cross-bridge state is designated C-state (Fig. 3A). (ii) When the tetanized fiber is stretched with a moderate velocity, a fraction of C-state cross-bridges are gradually brought into another state, in which they are displaced in the direction opposite to fiber shortening while attached to the thin filament, generate large tension, and are eventually detached from the thin filament without breaking ATP. Such a locked-on cross-bridge state is designated L-state (Fig. 3B); L-state cross-bridges are responsible for the development of tension response above the level of isometric tension generated by C-state cross-bridges. (iii) At the completion of stretch, L-state cross-bridges start returning to C-state, resulting in tension decay to the initial level of isometric tension (Fig. 3C). (iv) If

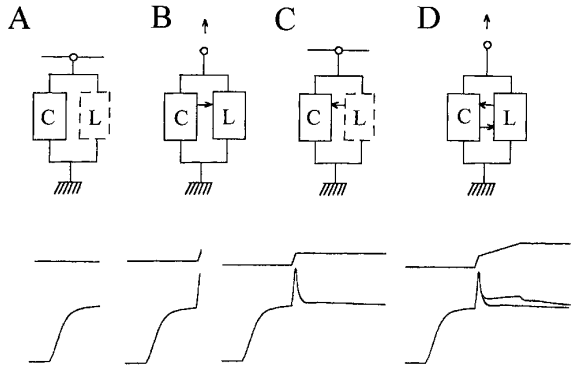

FIG. 3. Diagrams illustrating possible mechanism of algebraical summation of the component tension responses after a sudden reduction of stretch velocity. (A) When a tetanized muscle fiber is generating steady isometric tension, all the cross-bridges are in C-state. (B) When the isometrically tetanized fiber is stretched, a fraction of C-state cross-bridges are brought into L-state. L-state cross-bridges produce tension response to stretch above the isometric tension level. (C) After the completion of stretch, L-state cross-bridges start returning to C-state, resulting in the decay of tension response. (D) When stretch velocity is suddenly reduced above a certain extent, L-state cross-bridges, which have produced tension response, start returning to C-state, while at the same time a fraction of C-state cross-bridges are brought into L-state to produce the subsequent tension response.

stretch velocity is suddenly reduced above a certain extent, L-state cross-bridges, that have generated tension response start returning to C-state, while a fraction of C-state cross-bridges, that have generated isometric tension, are brought into L-state to produce the subsequent tension response. Thus, after a reduction of stretch velocity, the subsequent tension response starts on the falling tension baseline equal to the decay of the initial tension response (Fig. 3D).

During moderate-velocity stretches, C-state cross-bridges is assumed to maintain the steady level of isometric tension despite myofilament sliding (<1 μm/s) caused during stretch. In their cycling interaction with the thin filament, C-state cross-bridges may not remember their past history when once detached from the thin filament. If the rate of cross-bridge cycling in the isometric condition is assumed to be 20 s^{-1} (at 20°C) (10) and the fraction of time of attachment of a cross-bridge in each interaction cycle is assumed to be <0.05, then each attached cross-bridge is displaced by only less than 2.5 nm during myofilament sliding (<1 μm/s), a distance that may not seriously affect the mode of operation of C-state cross-bridges except for possible increase in tension generated by each cross-bridge, which may compensate the decrease in the number of C-state cross-bridges during stretch.

With respect to the mechanism how the change in the cross-bridge state is initiated, a possibility may be that C-state cross-bridges are brought into L-state if they are displaced by stretch while attached to the thin filament, while L-state cross-bridges may return to C-state if the velocity of their sliding along the thin filament is suddenly reduced above a certain extent. Cross-bridge states analogous to the above locked-on state are known in mammalian vascular smooth muscle (latch-state) (11) and molluscan catch muscle (catch-state) (12). Since the algebraical summation of tension responses appear to take place on the steady level of isometric tension, the number of C-state cross-bridges may always be much larger than that of L-state cross-bridges. This implies that, the tension response to stretch may be constructed above the isometric tension by a small number of L-state cross-bridges when they are forced to slide past the thin filaments.

With respect to the fraction of L-state cross-bridges attained after a moderate-velocity stretch, some considerations can be made based on the results of previous reports. Immediately after completion of a moderate-velocity stretch or isotonic lengthening, tetanized muscle fibers are known to shorten against a load equal to the maximum isometric tension P_0 (13, 14, 15) due to a shift of force-velocity curve towards higher force values, while the maximum unloaded velocity of shortening remains unchanged (13, 14). According to Cavagna *et al.* (15), this enhanced mechanical performance of the fiber continues for up to 20 ms, suggesting that, after the completion of stretch or lengthening, each L-state cross-bridge generates much higher time-averaged isometric tension than does each C-state cross-bridge. Under experimental conditions comparable with the present study (sarcomere length, 2.0-2.2 μm; extent of stretch or lengthening, $< 3\%$ of L_0), the maximum isometric force shifts from P_0 to about 1.1 P_0. Since the isometric force consists of algebraical sum of time-averaged tension generated by individual cross-bridges, this increase in P_0 may be expected to reflect the fraction of L-state cross-bridges attained at the end of stretch or lengthening; if, for example, time averaged isometric tension generated by each L-state cross-bridge is 5 times larger than that generated by each C-state cross-bridge, the fraction of L-state cross-bridges at the end of stretch or lengthening is estimated to be 0.025 ($1 \times 0.975 + 5 \times 0.025 = 1.1$).

A prediction derived from the forgoing mechanism is that the rate of increase of L-state cross-bridges in a tetanized fiber is much larger when a stretch with velocity V_2 is simply applied than when the same stretch is preceded by another stretch with velocity $V_1 > V_2$, since in the latter case L to C and C to L transitions take place simultaneously. It has been reported that the intensity of equatorial 1, 1 reflection ($I_{1,1}$) from a contracting frog skeletal muscle decreases during a moderate-velocity stretch (16). Recently, we have observed that the $I_{1,1}$ remains almost unchanged during a moderate-velocity stretch if it is applied immediately after the completion of a preceding stretch with a higher velocity (17). This result is consistent with,

though does not prove, the foregoing mechanism, if the decrease of the $I_{1,1}$ is assumed to reflect the increase in the number of L-state cross-bridges.

REFERENCES

1. Abbott, B. C., and Aubert, X. M. (1952) *J. Physiol.* **117,** 77–86.
2. Hill, A. V., and Howarth, J. V. (1959) *Proc. Roy. Soc.* **B151,** 169–193.
3. Curtin, N. A., and Davies, R. E. (1973) *Cold Spring Harb. Symp. Quant. Biol.* **37,** 619–626.
4. Huxley, A. F. (1957) *Prog. Biophys. Biophys Chem.* **7,** 255–318.
5. Huxley, H. E. (1960) *in* The Cell (Brachet, J., and Mirsky, A. E., Eds.), pp. 365–481, Academic Press, New York.
6. Sugi, H. (1972) *J. Physiol.* **225,** 237–253.
7. Gordon, A. M., Huxley, A. F., and Julian, F. J. (1966) *J. Physiol.* **184,** 170–192.
8. Gasser, H. S., and Hill, A. V. (1924) *Proc. Roy. Soc.* **B96,** 398–437.
9. Kobayashi, T., Kosuge, S., Shimada, M., and Sugi, H. (1996) *J. Muscle Res. Cell Motil.* **17,** 277–278.
10. Bagshaw, C. R. (1994) Muscle Contraction, Chapman & Hall, London.
11. Dillon, P. F., Aksoy, M. O., Driska, S. P., and Murphy, R. A. (1981) *Science* **211,** 495–497.
12. Twarog, B. M. (1976) *Physiol. Rev.* **56,** 829–838.
13. Edman, K. A. P., Elzinga, G., and Noble, M. I. M. (1978) *J. Physiol.* **281,** 139–155.
14. Sugi, H., and Tsuchiya, T. (1981) *J. Physiol.* **319,** 239–252.
15. Cavagna, G. A., Heglund, N. C., Harry, J. D., and Mantovani, M. (1994) *J. Physiol.* **481,** 689–708.
16. Amemiya, Y., Iwamoto, H., Kobayashi, T., Sugi, H., Tanaka, H., and Wakabayashi, K. (1988) *J. Physiol.* **407,** 231–241.
17. Kobayashi, T., Wakabayashi, K., Kosuge, S., and Sugi, H. (1997) *J. Muscle Res. Cell Motil.* **18,** 486.